Assistant Commissioner for Patents
Washington, DC 20231

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of

Osterfoff et al.		Atty. Ref.: 35-188
Serial No.	09/629,437	Group: 1646
Filed	July 13, 2000	Examiner: Ulm

For: EPIDIDYMIS-SPECIFIC RECEPTOR PROTEIN

DECLARATION UNDER RULE 132

I, Dr. Ulrich Gottwald, hereby declare as follows:

1. I am employee of Schering AG and presently hold the position of Senior Scientist. A copy of my CV is attached.

2. I have read the above-identified application, including the claims, and the Office Action dated July 18, 2001, issued in connection with the above-identified application.

3. It is my belief that one of ordinary skill in the art would appreciate that the above identified application identifies substantial and specific utilities for the protein of the above-identified application, DNA encoding the protein,

The following outlines the data presented herinafter and attached:

A. Construction of the knock out

B. Verification of the knock out

C. Knock out phenotype

C.1. Fertility phenotype/mating experiments

C.2. Spermatozoa phenotype

A. Construction of the knock out mouse

A knock out construction was generated using the exon/intron regions upstream of the beginning of the first transmembrane region and downstream of the seven transmembrane domain (7TM) for recombination (see fig.1). After successful recombination the whole 7TM of the murine counterpart of HE6 was deleted. The disrupted 7TM locus was replaced by a beta-galactosidase gene cassette (knock in), allowing the easy monitoring of the expression by means of simple X-Gal staining. LacZ was to be expressed expressed according to the HE6 expression pattern under control of the endogenous HE6 promotor.

Two positive HE6 KO lines (embryonic stem cell line E14) called A78 and A85 have been injected into C57/B16 blastocysts. After reimplantation into pseudopregnant females chimera animals have been obtained. Those with transmitted KO-construct into the germ line have been confirmed by PCR analysis (fig.2). The chimera males were than crossed with wild type

(WT) B16 female mice to produce heterozygous mice. Heterozygous females were mated with WT males to obtain hemizygous KO males with total loss of HE6. The HE6 gene had been mapped previously on the X chromosome in human. Some time later HE6 could be found in public domain databases mapped on position Xp21.3

No apparent lethality of HE6 knock out mice has been found. The relationship between born KO and WT males was as predicted by the Mendel law (fig. 3).

Two independent lines of HE6 KO mice, A78 and A85, have been derived and are used for fertility studies.

B. Verification of the knock out

Using the seven transmembrane region as a probe in the Northern blot no signal was observed in KO mouse (fig. 4).

HE6 protein can be localized by using specific polyclonal antisera derived from the immunization of rabbits with an N-terminal HE6 peptide. HE6 protein is present in WT mice on the kinocilia of epithelial cells of the ductuli efferentes and on the stereocilia of epithelial cells of the initial segment, the caput and partly on the corpus of the epididymis (fig. 9-16). Antibodies against the sequences of the present application therefore were used, as described in the present application, to analyze fertility. In HE6 KO

mice no kinocilia or stereocilia staining was recognizable with the antisera. The preimmune controls were also included.

In addition, the absence of the HE6 protein in KO mice was shown by Western blot analysis (fig. 5)

C. Knock out phenotype

Hemizygous KO males appeared normal and showed no obvious behavioral phenotype. Weight and size of the KO mice were comparable to WT mice. The organ weights of testis, epididymis, seminal vesicle, prostate, kidney, brain, spleen and heart were analyzed. No obvious differences were found when comparing eight KO with eight WT animals of the same litter. Only the weight of the epididymis is significantly reduced in KO male mice (fig. 6).

C.1 Fertility phenotype

The recent data from all the mating experiments is summarized in the attached Table 1. Sixteen KO mice derived from the A78 founder line and six KO mice from the A85 clone have been analyzed in up to three mating experiments.

Results with A78 KO mice: Animals aged up to nine weeks were infertile in 4 out of 13 experiments. In three cases they were dramatically subfertile (litters only one). Nine to twelve week old animals were infertile in

10 out of 15 experiments. Adult mice older than twelve weeks were infertile in 9 out of 13 experiments.

Results with A85 KO mice: similar results as seen with the A78 founder were obtained with the A85 KO mouse. Half of all animals up to 12 weeks were infertile (3/6). In experiments with mice older than 12 weeks 4 out of 6 were infertile.

These results clearly demonstrate that the function of HE6 is essential for male fertility. Even subadult animals have significantly reduced fertility. With increasing age KO mice tend to be infertile.

C.2 Spermatozoa phenotype

Spermatoza from the cauda epididymis were analyzed. In every case a prominent phenotype was observed. The sperm count was dramatically reduced. About 20% of the normal number of spermatozoa was found (fig.7). The observed motility is estimated at 0 and 1 according to the WHO-categorization.

8. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false tatements may jeopardize the validity of the application or any patent issuing thereon.

Dr. Ulrich Gottwald

Date: 31. January 2002